Eucalyptus: A Rich Source of Bioactive Secondary Metabolites

Inder Pal Singh

National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar- 160 062 (Punjab)

Eucalyptus is a rich source of biologically active compounds and is widely grown in different countries. The essential oil obtained from the leaves has medicinal value, while the secondary metabolites isolated from various species of *Eucalyptus* have shown a range of biological activities. Among the secondary metabolites produced by *Eucalyptus* are unique phloroglucinol-terpene adducts, which have not been isolated from other plants. Chemistry, biological activity and biogenesis of these metabolites are discussed in this review.

The genus *Eucalyptus* L' Heritier (Family Myrtaceae) originally native to Australia and now grown in over ninety countries is known for its fast growth[1]. Since ancient times, the bark and leaves of selected species have been used to treat colds, influenza, toothaches, snakebites, fevers, diarrhea and many other complaints. Kino, the astringent exudation produced after injury to wood, has been used in powdered form to treat open sores[1, 2]. *Eucalyptus* is considered a potent biomass in view of its rapid growth. The oil distilled from the leaves is used as an antiseptic and stimulant gargle. It is also used as an inhalant to clear catarrh. The diluted oil can be applied to chest as a decongestant and produces a warming effect. The aqueous extract of the leaves has been used in Chinese medicine to treat bacterial dysentery and pulmonary tuberculosis. Besides, being a rich source of terpenoids in the essential oil, *Eucalypts* also produce an array of unique biologically active secondary metabolites such as monomeric phloroglucinol compounds (grandinol, jensenone), phloroglucinol-terpene adducts (macrocarpals and euglobals), and dimeric phloroglucinols (grandinal and sideroxylonals). Flavanoids and stilbenes together with their glycosides, triterpenoids, and simple β-triketones have also been reported from this genus.

Essential oil

Eucalyptus is mainly grown for the production of essential oil whose medicinal potential was noted by the Europeans soon after their settlement in Australia. The primary component of the medicinal oil is 1,8-cineole, which should constitute at least 70% of the oil (British Pharmacopoeia). Industrial oils have piperitone and α-phellandrene as their major constituents. The other main components of the oil include citronellal, α-terpineol, α-eudesmol, β-eudesmol, α-pinene, β-pinene, carvone, aromadendrene, globulol, spathulenol, and guaiol. Besides these terpenoids, some cyclic β-triketones such as tasmanone, flavesone, leptospermone and agglomerone are also present in the essential oil[3]. The essential oil from *E. jensenii* is reported to contain at least sixty terpenes[4].

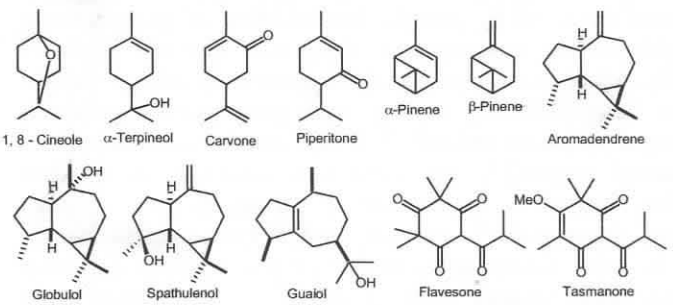

Figure 1: Some mono- and sesqui-terpenes in essential oil of *Eucalyptus*.

G-regulators

Eucalyptus species has produced a number of secondary metabolites belonging to diverse classes of natural products, and these metabolites have shown an interesting profile of biological activities. More extensively worked out among these metabolites are peroxy compounds G-inhibitors and phloroglucinol compounds. Three such growth regulators were isolated from mature leaves of *E. grandis* and named G-1 (**1**), G-2 (**2**), and G-3 (**3**)[5]. G-regulators showed promotory activity at low concentration (5×10^{-6} M) and inhibitory activity (5×10^{-4} M) at high concentration[6]. It was proposed that G regulators play a role in frost resistance as well as prevent water loss by effecting stomatal closure[7, 8]. However, synthetic

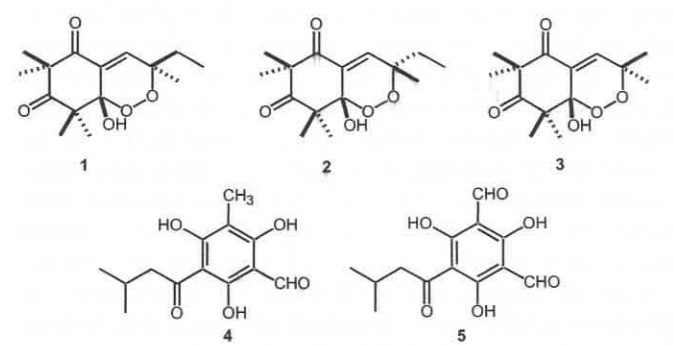

Figure 2: Structures of G-regulators and monomeric acylphloroglucinols.

G-regulators showed no inhibition of photosynthetic electron transport indicating the presence of some other more active component. Later work showed that monomeric phloroglucinol compounds grandinol and homograndinol were responsible for this inhibition of photosynthetic electron transport[9]. This underlines the fact that care should be exercised while evaluating natural products containing trace amounts of other impurities for biological activities.

Phloroglucinol compounds

Phloroglucinol compounds isolated from different species of *Eucalyptus* include monomeric and dimeric phloroglucinols as well as their adducts with mono- or sesqui-terpenes.

Monomeric phloroglucinols: The most studied monomeric phloroglucinol compound is grandinol (**4**) isolated from mature leaves of *E. grandis*. The structure of grandinol was established by spectroscopic, X-ray and synthetic studies[10, 11]. Grandinol has shown germination inhibitory activity, Epstein Barr Virus inhibitory activity, and photosynthetic electron transport inhibitory activity[9, 12]. It also inhibits transpiration and stomatal opening[13]. Grandinol also exhibited antibacterial activity against *B. subtilis* and *S. aureus* (5 µg/ml and 25 µg/ml, respectively)[14]. Another related compound jensenone (**5**) in which methyl group of grandinol is replaced by formyl group was isolated from *E. jensenii*[4]. Structurally, both grandinol and jensenone are assumed precursors of biologically active sideroxylonals, grandinal, euglobals, and macrocarpals.

Dimeric Phloroglucinols: Research in the laboratories of Kozuka et al and Etoh et al have focused on phloroglucinol compounds isolated from various species of *Eucalyptus* and this work has clearly shown the potential of this class of compounds. Sideroxylonals A-C (**6-8**) are novel dimeric phloroglucinol compounds found in the leaves and flower buds of some species of *Eucalyptus* genus[15, 16]. Sideroxylonals differ from each other in stereochemistry at C-7, C-10', and C-7'. The relative stereochemistry of sideroxylonal A was determined from the magnitude of coupling constant ($J_{7'-10'}$=11.7 Hz) and NOE between these protons. These compound have a wide range of biological effects, e.g. Sideroxylonals A and B showed anti-bacterial activity against Gram-positive bacteria *S. aureus* and *B. subtilis* at 3.9 and 7.8 µg/disk, respectively. They are strong inhibitors of human plasminogen activation[17]. Sideroxylonal A is also a potent attachment-inhibitor against marine fouling organism, blue mussel, *Mytilus edulis*[18]. They also limit the consumption of *Eucalyptus* leaves by koalas and other marsupial herbivores. They are potent mammalian anti-feedants as shown by feeding experiments on common ringtail possum and brushtail possum. This general herbivore ate lesser *Eucalyptus melliodora* when the concentration of sideroxylonal A was high[19]. Grandinal (**9**), which was isolated from *E. grandis* is also a dimeric phloroglucinol compound similar to sideroxylonals except an isovaleryl group replaces one formyl group[20]. Grandinal showed attachment inhibiting activity against the blue mussel. Grandinal was also tested for antibacterial activity against *S. aureus* and *B. subtilis*. The minimum inhibitory concentrations against these organisms were 50 and 100 µg/ml, respectively. More significant in grandinal is its existence as an inseparable mixture of three tautomers. Grandinal also possessed relative trans stereochemistry at positions 7'-10'.

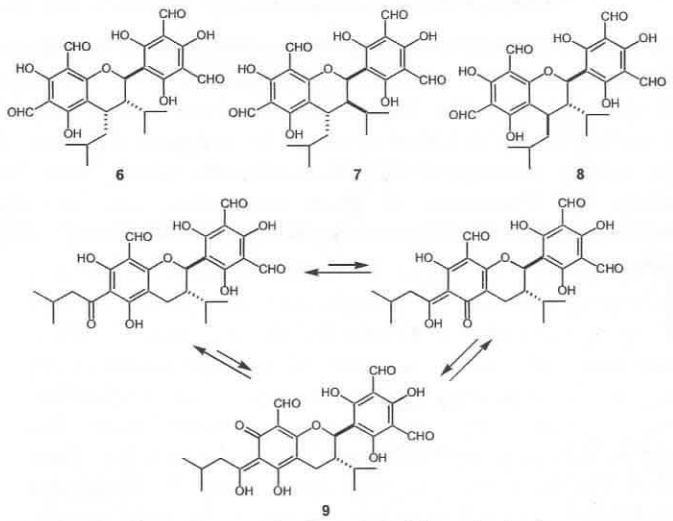

Figure 3: Structures of dimeric phloroglucinols.

Euglobals: Euglobals constitute the largest group of phloroglucinol-terpene adducts isolated from *Eucalyptus*, and are potent inhibitors of Epstein Barr Virus activation. Euglobals are diformyl or formyl-isovaleryl phloroglucinol-monoterpene or -sesquiterpene adducts. Euglobal III (**10**)[21], the first reported euglobal was isolated from *Eucalyptus globulus* LABILL. as a result of a search for potential granulation-inhibiting agents from among 1850 crude drugs. Since the initial report of euglobal III in 1980, a total of thirty-one euglobals have been isolated in the following years and their structures elucidated principally in the laboratories of Kozuka and Etoh[22-29]. The euglobals mainly differ structurally from each other in the nature of terpenoid moiety, with nine different terpenes (eight monoterpenes and a sesquiterpene) implicated in their formation. The euglobals can be subdivided into two groups, one containing monoterpene and the second containing sesquiterpene residue. Each of these can be further subdivided into two classes, one where acyl side chain of grandinol or jensenone precursor is involved in the formation of chroman ring and the second where it is not. The euglobals show strong inhibition of Epstein-Barr virus (EBV) activation induced by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA)[12, 30]. Some of the more recently isolated euglobals are G-6 (**11**), G-7 (**12**), G-8 (**13**), G-9 (**14**), G-10 (**15**), G-11 (**16**), and G-12 (**17**). Only a few representative structures are shown in the figure. From this class of compounds, euglobal G-1 (**18**) has shown cancer chemopreventive activity. It exhibited remarkable inhibitory effect on two-stage carcinogenesis test of mouse skin tumors induced by DMBA as an initiator and fumonisin B-1 as a

promoter. It also exhibited potent anti-tumor-promoting activity on two-stage carcinogenesis test of mouse pulmonary tumor using 4-nitroquinoline-N-oxide as an initiator and glycerol as a promoter[31]. The interest in these compounds is evidenced by numerous synthetic studies involving a variety of different routes. Robustadials A (**19**) and B (**20**), which were isolated as anti-malarial compounds from *E. robusta* also possess a skeleton similar to that of euglobals[32, 33].

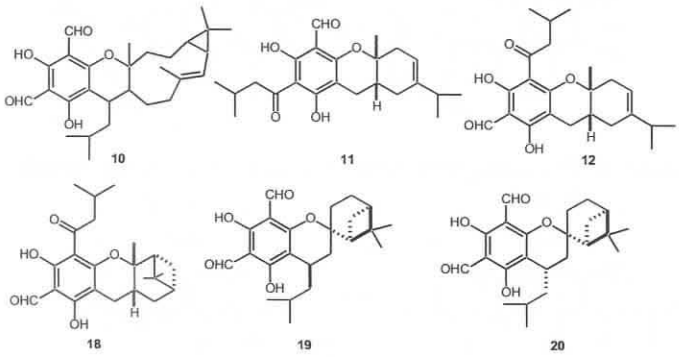

Figure 4: Structures of some euglobals.

Macrocarpals: Macrocarpals are phloroglucinol-terpene adducts and do not involve the formation of chroman ring as in eugobals. The first compound belonging to this series, macrocarpal A was isolated from *E. macrocarpa*[34]. Macrocarpal A (**21**) has diformyl-isopentyl phloroglucinol moiety joined to sesquiterpene globulol. This was followed by isolation of six more related compounds named macrocarpals B-G by the same group[35]. At the same time Nishizawa et al. isolated five related compounds from *E. globulus*[36]. One of these was identical to macrocarpal A, while the others were named M-B (**22**), M-C, M-D, and M-E. Macrocarpals A and B are stereoisomers at C-9'. More recently, macrocarpals H (**23**), I (**24**), J (**25**) were isolated from the leaves of *E. globulus*[37]. Macrocarpal H has the same aromatic moiety coupled to β-eudesmol having an exocyclic methylene at C-4. Macrocarpals I and J have β-methyl and α-OH at C-4 of the same sesquiterpene skeleton and are isomeric at C-9'. Macrocarpal-am-1 (**26**) was isolated from *E. amplifolia*, while macrocarpal K (**27**) was isolated from

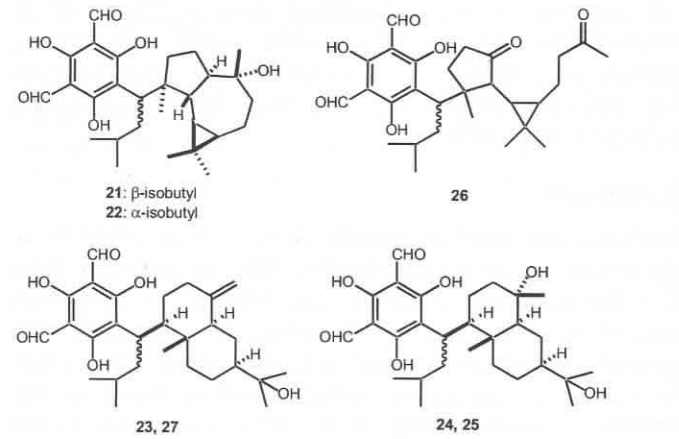

Figure 5: Structures of some macrocarpals.

E. globulus[38,39]. Macrocarpals have shown a range of biological activities, they are active against Gram-positive bacteria[35] and periodontopathic bacteria[37], inhibited HIV reverse transcriptase[36] and aldose reductase[40], and showed attachment inhibitory activity against the blue mussel, *Mytilus edulis*[41].

Sideroxylonals, grandinal, euglobals, and macrocarpals are unique to *Eucalyptus* genus, although within this genus they are found in a number of species.

Biogenetic aspects of phloroglucinol compounds

The biogenetic origin of phloroglucinol compounds can be traced to polyketides that arise from polyketomethylene chains [-(CH_2-CO)m-]. There is a close parallel between biosynthesis of fatty acids and polyketides, since, in both cases formation of linear chains proceeds by addition of C_2 units. However, in fatty acid biosynthesis every C_2 unit is added to growing chain only after the reduction of previous carbonyl group to a methylene group. The growth of polyketide chain requires no such prior reduction. Instead, poly-β-keto acids are formed which are highly reactive since they contain both active methylene groups (potential nucleophiles) and carbonyl groups (potential electrophiles).

In case of acyl phloroglucinol compounds in *Eucalyptus*, different alkanoyl coenzyme A starter units are extended by three malonyl coenzyme A units and the polyketide species thus generated undergo a Claisen type cyclization reaction to yield precursor to acyl phloroglucinol. The active form of the first C_2 unit is acetyl CoA, while malonyl CoA formed by carboxylation of acetyl CoA is used for subsequent C_2 units. Monomethylation of doubly activated carbons by *S*-adenosyl-methionine results in the introduction of nuclear methyl groups. Biosynthetic studies on tasmanone have confirmed the origin of nuclear methyl groups. Further, monomethylation of at least one nuclear carbon prevents tautomerization of phloroglucinol ring to triketone. The phenolic hydroxyls and the carbonyls thus end up in alternate positions giving a typical oxygenation pattern to polyketide compounds. Nuclear methyl groups undergo successive oxidation reactions to generate carboxyl groups. These oxidations thus lead to grandinol and jensenone (figure 6)[42].

Dimeric compounds, sideroxylonals and grandinal can be derived biogenetically from grandinol and jensenone. Biogenetic route for grandinal is shown in figure 7. *o*-Quinone methide (**28**) generated from grandinol and styrene compound (**29**) generated from jensenone can undergo hetero Diels-Alder reaction to generate the chroman ring of grandinal. It should be mentioned, however, that none of these intermediates have yet been isolated from *Eucalyptus*. However, biomimetic synthesis of grandinal and euglobals utilizing Diels-Alder cycloaddition lend support to the biogenetic theories.

The biogenesis of macrocarpals is proposed to involve carbocationic species (**30**) derivable from (**4**). Carbocation (**30**) can act as initiator for the cyclization of sesquiterpene

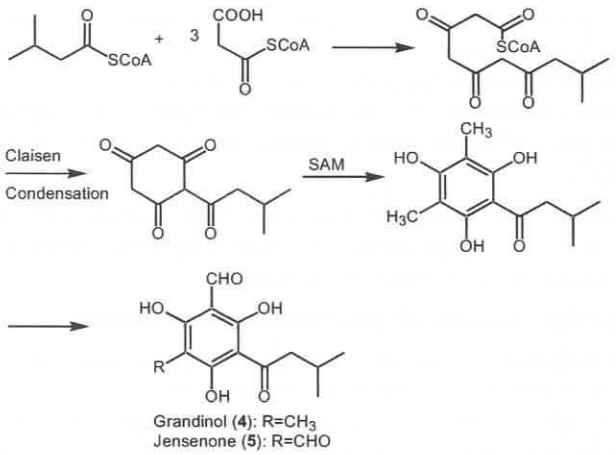

Figure 6: Proposed biogenetic pathway of grandinol (4) and jensenone (5).

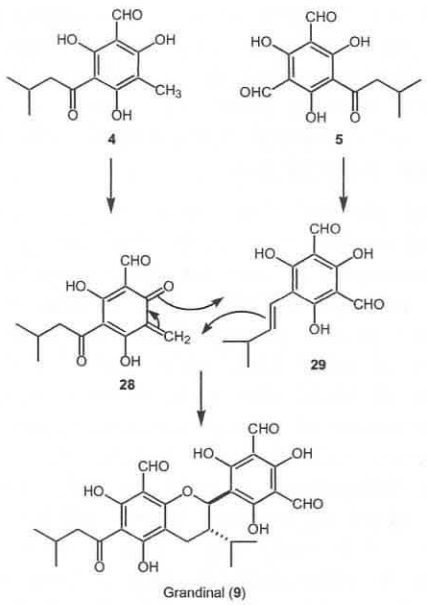

Figure 7: Proposed biogenetic pathway of grandinal (9).

precursors to give macrocarpals. The proposed biogenesis of macrocarpal-am-1 is shown in figure 8. Sesquiterpene bicyclogermacrene **31** can cyclize in the presence of **30** to generate globulol or aromadendrane skeleton of macrocarpals A and B. Macrocarpal-am-1 may be formed by dehydration of macrocarpal A or B generating a tetrasubstituted double bond at $C_{1\text{-}10}$ which can then undergo oxidative cleavage to give macrocarpal-am-1 (**26**). Biogenesis of euglobals G6 (**11**) and G7 (**12**) can be similarly rationalized by assuming the intermediacy of two regioisomeric *o*-quinone methides derived from grandinol. A [4+2] cycloaddition reaction between *o*-quinone methides and γ-terpinene would generate these euglobals (figure 9).

Other miscellaneous compounds

Apart from this unique class of phloroglucinol-terpene derived secondary metabolites, several other unexceptional

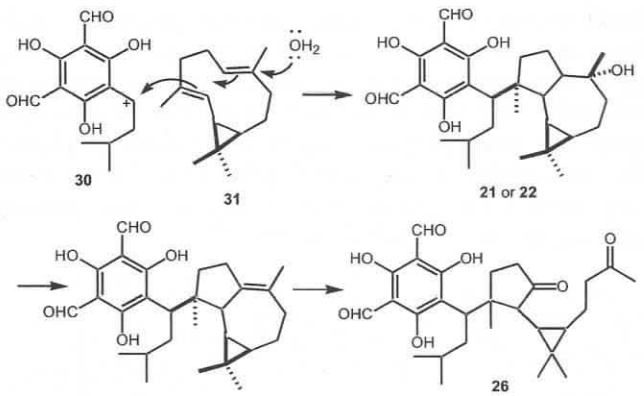

Figure 8: Proposed biogenetic pathway of macrocarpal-am-1 (26).

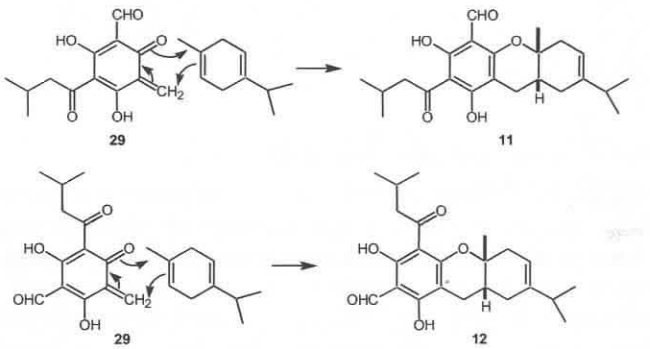

Figure 9: Proposed biogenetic pathway of euglobals 11 and 12.

compounds such as flavanoids and their glycosides, triterpenoids, long chain diketones etc. have also been reported from *Eucalyptus* species. Flavanoids sideroxylin, 8-desmethylsideroxylin and eucalyptin were isolated from several species of *Eucalyptus*[43-45], kaempferol, quercetin, ellagic acid, gallic acid and gentisic acid were reported from *E. sideroxylon*[46]. Resinosides possessing monoterpene carboxylic acid and substituted flavanoid glucoside moiety were isolated from *E. resinifera* and stilbene glucosides were isolated from *E. rubida* as repellents against the blue mussel[47,48]. Long chain β-diketone, *n*-tritriacontan-16,18-dione was isolated as an anti-oxidant from leaf wax of *E. globulus*[49]. Several triterpenes such as ursolic acid lactone, oleanolic acid, maslinic acid, camaldulic acid, camaldulensic acid, and camaldulenic acid etc. have been isolated from *Eucalyptus* species[50].

Conclusions

Eucalyptus has been extensively studied for its essential oil and unique secondary metabolites. Although essential oil is used for medicinal purposes, the potential of secondary metabolites obtained from *Eucalyptus* has not been fully explored. Several unique metabolites have been isolated from the foliage of *Eucalyptus*, efforts need to made in this direction to develop some of the more active compounds so that secondary industry, in addition to the extraction of

essential oil and use of wood, can be developed to make use of the leaves of this plant.

References

1. Elliot, W. R. and Jones, D. L. (1984), Encyclopedia of Australian Plants Suitable for Cultivation, Vol. 4. Lothian Publications, Melbourne.
2. Barr, A. (1988), Traditional Bush Medicine: An Aboriginal Pharmacopoeia. Greenhouse Publications, Richmond, Victoria.
3. Boland, J. et al. (1991), Eucalyptus Leaf Oils: Use, Chemistry, Distillation and marketing. ACIAR/CSIRO, Australian Center for International Agricultural Research, Inkata Press, Melbourne.
4. Boland, J. et al. (1992), Phytochem. 31: 2178-2179.
5. Crow, W. D. et al. (1971), Tetrahedron Lett. 1353-1356.
6. Dhawan A. K. et al. (1979), Planta, 146: 419-422.
7. Paton, D. M. (1981), Aust. J. Chem. 29: 675-688.
8. Paton, D. M. et al. (1980), Plant Physiol. 66: 254-256.
9. Yoshida, S. et al. (1988), Phytochem. 27: 1943-1946.
10. Crow, W. D. et al. (1977), Tetrahedron Lett. 1073-1074.
11. Yuste, F. et al. (1978), Tetrahedron Lett. 4869-4870.
12. Takasaki, M. et al. (1990), Chem. Pharm. Bull. 38: 2737-2739.
13. Yoneyama, K. et al. (1996), Plant Growth Regulation. 19: 7-11.
14. Nakayama, R. et al. (1990), Agric. Biol. Chem. 54: 231-232.
15. Satoh, H. et al. (1992), Chem. Lett. 1917-1920.
16. Eschler, B. M. and Foley, W. J. (1999), Aust. J. Chem. 52: 157-158.
17. Neve, J. et al. (1999), J. Nat. Prod. 62: 324-326.
18. Singh, I. P. et al. (1996), Biosci. Biotech. Biochem. 60: 1522-1523.
19. Wallis, I. R. et al. (2002), Aust. J. Zool. 50: 507-519.
20. Singh, I. P. et al. (1997), Biosci. Biotech. Biochem. 61: 921-923.
21. Sawada, T. (1980), Chem. Pharm. Bull. 28: 2546-2548.
22. Kozuka, M. et al. (1982), Chem. Pharm. Bull. 30: 1952-1963.
23. Kozuka, M. (1982), Chem. Pharm. Bull. 30: 1964-1973.
24. Takasaki, M. et al. (1990), Chem. Pharm. Bull. 38: 1444-1446.
25. Kokumai, M. et al. (1991), J. Nat. Prod. 54: 1082-1086.
26. Takasaki, M. et al. (1994), Chem. Pharm. Bull. 42: 2177-2179.
27. Takasaki, M. et al. (1994), Chem. Pharm. Bull. 42: 2113-2116.
28. Singh, I. P. et al. (1998), Phytochem. 47: 1157-1159.
29. Umehara, K. et al. (1998), Phytochem. 49: 1699-1704.
30. Takasaki, M. et al. (1995), Biol. Phar. Bull. 18: 435-438.
31. Takasaki, M. et al. (2000), Cancer Lett. 155: 61-65.
32. Xu, R. et al. (1984), J. Am. Chem. Soc. 106: 734-736.
33. Cheng, Q. and Snyder, J. K. (1988), J. Org. Chem. 53: 4562-4567.
34. Murata, M. et al. (1990), Agric. Biol. Chem. 54: 3221-3226.
35. Yamakoshi, Y. et al. (1992), Biosci. Biotech. Biochem. 56: 1570-1576.
36. Nishizawa, M. Et al. (1992), Tetrahedron Lett. 33: 2983-2986.
37. Osawa, K. and Yasuda, H. (1996), J. Nat. Prod. 59: 824-827.
38. Singh, I. P. and Etoh. H. (1995), Biosci. Biotech. Biochem. 59: 2330-2332.
39. Singh, I. P. et al. (1999), Nat. Prod. Lett. 14: 11-15.
40. Murata, M. et al. (1992), Biosci. Biotech. Biochem. 56: 2062-2063.
41. Singh I. P. and Etoh, H. (1997), Nat. Prod. Sci. 3: 1-7.
42. Ghisalberti, E. (1996), Phytochem. 41: 7-22.
43. Wollenweber, E. and Kohorst, G. (1982), Z. Naturforsch. 36c:913-915.
44. Cardona, M. L. and Seoan, E. (1982), Phytochem. 21: 2759-2760.
45. Horn, D. H. S. and Lamberton, J. A. (1963), Chem. And Ind. 691-692.
46. Hillis, W. E. and Isoi, K. (1965), Phytochem. 4: 541-550.
47. Hyodo, S. et al. (1992), Biosci. Biotech. Biochem. 56: 138.
48. Etoh, H. et al. (1990), Agric. Biol. Chem. 54: 2443-2444.
49. Osawa, T. and Namiki, M. (1981), Agric. Biol. Chem. 45: 735-739.
50. Begum, S. et al. (1997), J. Nat. Prod. 60: 20-23.